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COMBINED THERAPY OF SEPTICEMIA WITH OFLOXACIN AND/OR SYNTHETIC TREHALOSE DICORYNOMYCOLATE (S-TDCM)IN IRRADIATED AND WOUNDED MICE

DIE KOMBINIERTE THERAPIE DER SEPTIKÄMIE MIT OFLOXACIN UND/ODER SYNTHETISCHEM TREHALOSE-DICORYNOMYCOLAT (S-TDCM) BEI BESTRAHLTEN UND VERWUNDETEN MÄUSEN

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SUMMARY

Following lethal irradiation, mice usually succumb to sepsis as a result of translocation of intestinal bacteria and impairment of the host defense system. Additional trauma in these immunocompromised mice further increases susceptibility to bacterial infection from either endogenous or exogenous origin. Treatment with ofloxacin or synthetic trehalose dicorynemycolate (S-TDCM) and was evaluated in mice, which were lethally irradiated and wounded, and which died with sepsis within six days. Wounding was performed on C3H/HeN mice anesthetized by inhalation of methoxyfurane. Dorsal skin and muscle equal to 30% total body surface was removed 1 h after 8.0 Gy gamma radiation. S-TDCM, which augments nonspecific resistance to infection in irradiated mice, was given once i.p. immediately after wounding. Ofloxacin was injected s.c. daily from day 0 to day 10. *Staphylococcus aureus*, *Streptococcus faecium*, and *Escherichia coli* were isolated from both the livers and wound sites of moribund, untreated mice 4 and 5 days postirradiation. Although all mice died, ofloxacin increased the mean survival time from 4.7 days (untreated) to 11.4 days and decreased the number of bacterial species isolated from liver and wound. Combined treatment with ofloxacin and S-TDCM did not increase survival time compared with ofloxacin treatment alone. Although they prolong survival, ofloxacin and S-TDCM alone are inadequate for effective therapy of polymicrobic infections in irradiated/wounded mice.

ZUSAMMENFASSUNG

Im Gefolge letaler Bestrahlung fallen Mäuse normalerweise einer Sepsis zum Opfer, bedingt durch die Translokation intestinaler Bakterien und der Dysfunktion des wirtseigenen Abwehrsystems. Die Empfänglichkeit gegenüber bakteriellen Infektionen wird bei solchermaßen immunkomprimittierten Mäusen durch zusätzliche Wundtraumata entweder endogenen oder exogenen Ursprungs weiter erhöht. Bei letal bestrahlten und verwundeten Mäusen, die innerhalb von 6 Tagen an Sepsis starben, wurde die Behandlung mit Ofloxacin und/oder synthetischem Trehalose-Dicorynomycolat (S-TDCM) überprüft. Das Setzen von Wunden wurde an C3H/HeN-Mäusen vorgenommen, die durch die Inhalation von Methoxyfluran anästhetisiert wurden. Eine Stunde nach 8.0 Gy Gamma-Bestrahlung wurden dorsal Haut- und Muskelgewebe in Entsprechung von 30% der gesamten Körperoberfläche entfernt. Unmittelbar

nach der Wundsetzung wurde S-TDCM, welches die unspezifische Resistenz gegenüber Infektionen bei bestrahlten Mäusen erhöht, in einer einmaligen Dosis intraperitoneal appliziert. Ofloxacin wurde täglich subkutan von Tag 0 bis Tag 10 injiziert. *Staphylococcus aureus, Streptococcus faecium* und *Escherichia coli* wurden sowohl aus den Lebern, als auch von den Wundstellen sterbender, unbehandelter Mäuse im Zeitraum von 4 und 5 Tagen nach der Bestrahlung isoliert. Obwohl alle Mäuse starben, erhöhte Ofloxacin die mittlere Überlebenszeit von 4,7 Tagen (unbehandelt) auf 11,4 Tage und erniedrigte die Anzahl der aus Leber und Wunden isolierten Bakterienspezies. Die kombinierte Behandlung mit Ofloxacin und S-TDCM erhöhte die Überlebenszeit im Vergleich mit alleiniger Ofloxacin-Behandlung nicht. Ofloxacin und S-TDCM alleine sind für die effektive Therapie von Infektionen durch mehrere Mikroben bei bestrahlten und verwundeten Mäusen ungeeignet, obwohl sie die Überlebenszeit verlängern.

INTRODUCTION

One of the many problems that physicians will encounter in the treatment of radiation victims is providing life-sustaining support to individuals who have not only received a lethal dose of radiation but have received physical trauma as well. We previously showed that i.p. injection of synthetic trehalose dicorynemycolate (S-TDCM) 20 h before or 1 h after lethal irradiation significantly increases survival in B6D2F1 or C3H/HeN mice (MADONNA et al., 1989). Further, S-TDCM-enhancement of survival in irradiated mice was shown to be associated with a reduction in sepsis, increase in nonspecific resistance to infection, and stimulation of hematopoesis. Ofloxacin is one of a new generation of fluorinated quinolones with activity against most Gram-negative bacteria, many Gram-positive bacteria and some anaerobes (MONK and CAMPOLI-RICHARDS, 1987).

Our purpose in the present study was to determine whether S-TDCM or ofloxacin would increase survival in irradiated mice that are wounded 1 h after irradiation and which die one week earlier than mice exposed to radiation alone. Results showed that aggressive antibiotic therapy with ofloxacin, but not S-TDCM injection, significantly increased the survival time of combined injured mice. In addition, ofloxacin therapy reduced the number of bacterial species cultured from the wound and liver of irradiated wounded mice.

METHODS

Radiation

C3H/HeN female mice (8 – 12 weeks) were given a whole body dose of ^{60}Co radiation (MADONNA et al., 1989). Mice received 8.0 Gy at 0.4 Gy/min. This dose results in 85 – 100% mortality in 30 days.

Wounding and S-TDCM Therapy

One hour postirradiation, groups of mice were anesthetized by inhalation of methoxyflurane and treated as follows:

Irradiated Group

Mice were injected with a suspension of S-TDCM (Ribi ImmunoChem Research, Inc., Hamilton, MT) (200 μg/mouse, i.p.) in 0.2% Tween 80/saline, or saline (control) (0.5 ml/mouse, i.p.). The synthesis and preparation of S-TDCM has been described (MADONNA et al., 1989).

Combined-Injury Group

Mice were given a 30% total-body-surface-area wound (LEDNEY et al., 1985) and then injected i.p. with either S-TDCM or saline (control). The time required for the wound trauma procedure is approximately 10 minutes. In brief, the fully anesthetized mouse is subjected to wound trauma by punching out a double layer of dorsal surface skin between the shoulders. The panniculus carnosus muscle and overlying skin is removed by sliding the loose dorsal skin away from the body and by striking a steel punch with a hammer. The procedure is done on a clean teflon covered operation board. Aseptic technique is used throughout the entire procedure. The wound size is 30% of the total skin surface.

Antibiotic Therapy

Ofloxacin (Ortho Pharmaceuticals) was prepared in sterile, pyrogen-free water and was injected into mice (0.1 ml/mouse, s.c.) at a dose of 40 mg/kg/day. Control mice received 0.1 ml of water/day, s.c.

Isolation of Bacteria From Wound Site and Liver

Additional mice were included in each group to assess the degree of bacterial colonization of the wound and/or the presence of sepsis indicated by bacteria in the liver.

Wound Culture

The wound site of injured mice was cultured for bacteria by rolling a sterile, saline-soaked cotton swab over the wound surface. The swab was then inoculated to one plate of 5% defibrinated sheep blood agar (with phenyl ethanol) and one plate of MacConkey Agar. Media were incubated overnight at 35 C. Colonies of bacteria were identified by standard diagnostic procedures (ASM Manual of Clinical Laboratory Microbiology).

Liver culture

Mice were euthanized by cervical dislocation and a portion of each liver was aseptically removed, weighed, and homogenized in cold saline solution. Serial dilutions of each liver homogenate were made in cold saline solution and suspensions were inoculated in duplicate to media by a Spiral plater (Spiral Systems Inc.), enumerated and identified.

RESULTS

Survival and Culture of Irradiated/Wounded Mice

Ofloxacin therapy increased the mean survival time of irradiated/wounded mice from 4.7 days (untreated) to 11.4 days (treated) (Figure 1). Culture of the wounds of untreated, moribund mice on day 4 yielded *Staphylococcus aureus*, *Streptococcus faecium*, *Escherichia coli*, and *Proteus mirabilis*. In contrast, only *S. aureus* and *S. faecium* were recovered from the wounds of ofloxacin-treated mice. In addition, *P. mirabilis* was recovered from the livers of untreated mice on day 4, whereas no bacteria were recovered from the livers of irradiated wounded mice treated with ofloxacin.

Although S-TDCM increased the survival of irradiated mice, it decreased the mean survival time of irradiated wounded mice from 4.7 days to 3.8 days. Cultures of the wounds of irradiated/wounded mice yielded *S. aureus*, *S. faecium*, *E. coli*, and *P. mirabilis* as did those of untreated mice. *P. mirabilis* was isolated from the livers of both untreated and treated mice.

Combination therapy with S-TDCM and ofloxacin decreased survival time of irradiated/wounded mice compared with ofloxacin treatment alone by 1.1 days (11.4 days with ofloxacin vs. 10.2 days with ofloxacin and S-TDCM). No bacteria grew in liver cultures from ofloxacin-treated mice 4 days postirradiation. In contrast, cultures of liver from S-TDCM/ofloxacin treated mice yielded *S. faecium* and *S. aureus*.

DISCUSSION

These studies demonstrated that early mortality in combined injured mice is associated with fulminant infection most likely resulting from the unhindered spread of bacteria from the wound site. The usefulness of aggressive antibiotic therapy to suppress the growth and spread of opportunistic bacteria was demonstrated by the increased mean survival time and the reduction in the number of bacterial species isolated from the wound and liver of ofloxacin-treated mice.

Results suggested that therapy with an immunomodulator, such as S-TDCM, can be toxic rather than beneficial. Interestingly, previous studies have shown that combined therapy with natural TDM from *Mycobacterium phlei* and ceftriaxone (a third-generation cephalosporin) synergistically increased survival of mice exposed to a sublethal dose of radiation and challenged with a lethal dose of *Klebsiella pneumoniae* (MADONNA et al., 1989).

These studies illustrated the association and complexity of wound trauma and susceptibility to infection in the immunocompromised host. Further investigations into the mechanisms of early mortality in combined injury will be necessary in order to determine better treatment regimens.

Experiments are in progress to determine whether ceftriaxone is more efficacious than ofloxacin when combined with S-TDCM therapy in irradiated/wounded mice. The spectrum of activity of ceftriaxone includes staphylococci, except methicillin-resistant strains, but not enteric streptococci.

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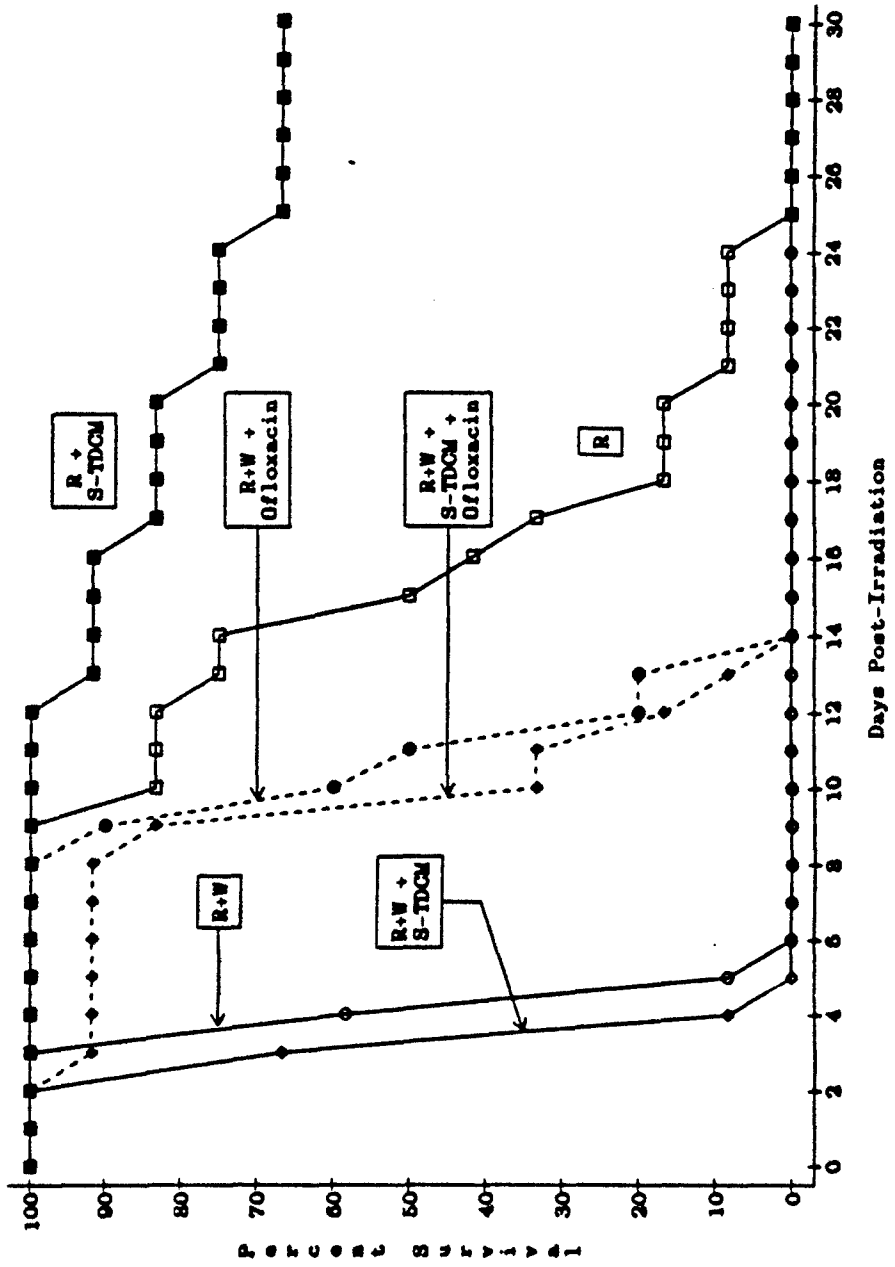

Figure 1: Survival of irradiated/wounded mice given S-TDCM and treated with ofloxacin. Mice (N = 12) were irradiated/wounded (R + W) or irradiated alone (R) on day 0. Mice in each group were injected with S-TDCM (200 μg/mouse, i.p.) or saline solution 1 h post-irradiation. Ofloxacin or water (control) was injected s.c. (40 mg/kg/day) on days 0–10. P values determined by the generalized Savage (Mantel Cox) method were: P = 0.0035 for R + W vs. R + W(S-TDCM), P < 0.001 for R vs. R(S-TDCM), and P > 0.05 for R + W(ofloxacin) vs. R + W(S-TDCM + Ofloxacin).

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LITERATURE

LEDNEY, G.D., STEWART, D.A., GRUBER, D.F., GELSTON, H.M., EXUM, E.D., and SHEERY, P.A.: Hematopoietic Colony-Forming Cells from Mice After Wound Trauma. J. Surg. Research. *38*, 55-65 (1985).

MADONNA, G.S., LEDNEY, G.D., ELLIOTT, T.B., BROOK, I., ULRICH, J.T., MYERS, K.R., PATCHEN, M.L., and WALKER, R.I.: Trehalose Dimycolate Enhances Resistance to Infection in Neutropenic Animals. Infect. Immun. *57*, 2495-2501 (1989).

MONK, J.P., and CAMPOLI-RICHARDS, D.M.: Ofloxacin: A Review of its Antibacterial Activity, Pharmacokinetic Properties and Therapeutic Use. Drugs *33*, 346-391 (1987).